APPENDIX C

ORIGINAL ARTICLE

Autoantibodies against epidermal transglutaminase are a sensitive diagnostic marker in patients with dermatitis herpetiformis on a normal or gluten-free diet

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Background: Dermatitis herpetiformis (DH) is a cutaneous manifestation of gluten-sensitive enteropathy (celiac disease). Patients with DH demonstrate circulating IgA antibodies against epidermal transglutaminase (eTG) and tissue transglutaminase (tTG). It has been suggested that eTG is the autoantigen of DH.

Objective: The purpose of this study was to characterize the autoimmune response to eTG and tTG in patients with DH on a normal or gluten-free diet (GFD).

Methods: Sera from 52 patients with DH were studied for the presence of IgA antibodies to eTG and tTG by enzyme-linked immunosorbant assay. In 38 patients, serum was obtained before initiation of a GFD, whereas 14 patients had been on a GFD for at least 2 years.

Results: Autoantibodies against eTG were detected in 36 of 38 patients (95%) and those against tTG in 30 of 38 patients (79%) with DH on a normal diet. Of 14 patients on a long-term GFD, 7 patients were free of DH lesions and did not require dapsone treatment. None of these patients showed circulating antibodies against eTG or tTG. The remaining 7 patients on a GFD were not able to stop taking dapsone. All these patients demonstrated anti-eTG antibodies, whereas only 3 of them showed additional reactivity against tTG.

Limitation: Autoantibody levels against eTG and tTG before and after introduction of a GFD were not examined in the same patients.

Conclusion: Our data suggest that antibodies to eTG are the most sensitive serologic marker in treated and untreated patients with DH and confirm the central role of eTG in the pathogenesis of this disease. (J Am Acad Dermatol 10.1016/j.jaad.2008.12.037.)

Key words: dermatitis herpetiformis; epidermal transglutaminase; gluten-sensitive enteropathy; tissue transglutaminase.

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Gluten-sensitive enteropathy (GSE), also referred to as celiac disease or celiac sprue, is a state of increased immunologic responsiveness to ingested gliadin proteins from wheat, barley, and rye, leading to malabsorption. Dermatitis herpetiformis (DH) is a cutaneous manifestation of GSE. It is an intensely pruritic subepidermal blistering disorder predominantly affecting extensor surfaces of major joints and characterized by granular deposits of IgA in the papillary dermis.[1]

Patients with DH and GSE have circulating IgA antibodies against endomysium, targeting tissue transglutaminase (tTG).[2-5] The levels of anti-tTG antibodies correlate with the degree of intestinal damage and decrease under a gluten-free diet

Abbreviations used:

DH: dermatitis herpetiformis
ELISA: enzyme-linked immunosorbent assay
eTG: epidermal transglutaminase
GFD: gluten-free diet
GSE: gluten-sensitive enteropathy
tTG: tissue transglutaminase

(GFD).[5-7] Therefore, levels of tTG-specific antibodies serve as a useful indicator of patient adherence to a GFD.

Epidermal transglutaminase (eTG) is an enzyme expressed in the epidermis that is homologous but not identical to tTG. Patients with DH produce two IgA antibody populations against eTG. The first population binds exclusively eTG, whereas the second one cross-reacts with both eTG and tTG.[8] The cross-reactive eTG-specific antibodies are found in GSE without DH as well but demonstrate a lower avidity for eTG than in patients with DH. In contrast, eTG-specific antibodies noncross-reactive with tTG are found only in patients with DH.[8] Furthermore, eTG but not tTG colocalizes with granular IgA deposits in the skin of patients with DH[8,9] and levels of antibodies against eTG correlate with the extent of enteropathy in DH but not in GSE without DH.[10] Taken together, these data suggest that eTG rather than tTG is the autoantigenic target in patients with DH.

In this study, we evaluate the prevalence of IgA antibodies to eTG and tTG in a large cohort of patients with DH and demonstrate that eTG-specific antibodies may be the only serologic marker of this disease in patients on either a normal diet or a GFD.

MATERIALS AND METHODS

Patients

Serum samples were obtained from 52 patients with DH, whose demographic data are summarized in Tables I and II. The diagnosis of DH was confirmed by direct immunofluorescence microscopy of perilesional skin biopsy specimens, showing granular deposits of IgA in the dermal papillae and/or along the dermoepidermal junction in all cases. In 38 patients, serum samples were collected before initiation of treatment (Table I), whereas the remaining 14 patients were on a GFD (Table II). Only patients after a GFD for at least 2 years were included in the latter group. The diagnosis of underlying GSE was confirmed by a small intestinal biopsy specimen in 30 patients (18 untreated and 12 treated) and classified according to the Marsh criteria.[11] Patients receiving a GFD were further divided into two subgroups. The first subgroup, consisting of 7 patients, was completely free of DH lesions under a GFD and did not require any maintenance dapsone treatment. The second subgroup of further 7 patients was unable to stop taking dapsone while on a GFD because of repeated recurrence of pruritic DH lesions (Table II). One of these patients developed DH after 2 years of a strict GFD treatment administered for biopsy-proven GSE. The study was approved by the local ethics committee and informed consent was obtained from all patients.

Table I. Epidermal and tissue transglutaminase reactivity and intestinal biopsy findings in patients with dermatitis herpetiformis on a normal diet

Patient No.	Sex/age, y	eTG ELISA, U/mL	tTG ELISA, U/mL	Marsh type
1	M/58	49	11	I
2	M/46	12	16	IIIa
3	M/26	41	36	IIIa
4	F/55	246	226	IIIa
5	M/72	151	165	IIIa
6	M/58	152	100	IIIa
7	M/68	111	55	IIIa
8	F/41	69	42	IIIa
9	F/26	29	56	IIIa
10	F/29	52	71	IIIa
11	M/73	106	55	IIIb
12	F/70	97	41	IIIb
13	F/59	3	39	IIIb
14	M/66	63	43	IIIb
15	M/61	214	118	IIIb
16	F/46	166	155	IIIb
17	M/26	39	129	IIIb
18	M/65	25	7	IIIb
19	M/50	108	53	Unknown
20	F/71	103	8	Unknown
21	M/85	162	10	Unknown
22	F/65	67	10	Unknown
23	F/69	77	127	Unknown
24	F/67	126	25	Unknown
25	M/27	91	129	Unknown
26	M/38	60	119	Unknown
27	F/75	53	53	Unknown
28	F/65	34	56	Unknown
29	M/43	82	117	Unknown
30	M/28	58	40	Unknown
31	M/54	39	28	Unknown
32	F/35	66	54	Unknown
33	F/53	34	34	Unknown
34	M/42	41	3	Unknown
35	M/67	27	38	Unknown
36	M/36	215	204	Unknown
37	F/32	40	36	Unknown
38	M/63	39	12	Unknown

ELISA, Enzyme-linked immunosorbent assay; *eTG*, epidermal transglutaminase; *F*, female; *M*, male; *tTG*, tissue transglutaminase. Cut off for both ELISA systems is 18 U/mL.

Table II. Epidermal and tissue transglutaminase reactivity and dapsone dose in patients with dermatitis herpetiformis on a gluten-free diet

Patient No.	Sex/age, y	eTG ELISA, U/mL	tTG ELISA, U/mL	Dapsone, mg/d	Disease duration, y	Marsh type*
			Patients without dermatitis herpetiformis lesions			
1	F/46	10	10	None	2	IIIb
2	M/46	6	4	None	8	Unknown
3	F/18	16	13	None	27	IIIc
4	M/30	6	5	None	3	IIIa
5	M/25	17	14	None	2	IIIb
6	M/61	12	8	None	4	IIIb
7	M/36	13	12	None	2	IIIb
			Patients with dermatitis herpetiformis lesions			
1	M/85	58	44	25	25	Unknown
2	M/34	27	17	100	11	I
3	M/71	39	24	100	4	IIIc
4	F/53	23	14	50	3	IIIa
5	F/72	39	16	100	3	I
6	F/72	36	23	25	25	IIIa
7	M/44	38	15	25	2	IIIa

ELISA, Enzyme-linked immunosorbent assay; *eTG*, epidermal transglutaminase; *F*, female; *M*, male; *tTG*, tissue transglutaminase.
Cut off for both ELISA systems is 18 U/mL.
*Marsh type was determined before introduction of a gluten-free diet.

Enzyme-linked immunosorbent assay

IgA reactivity to eTG and tTG was assayed using commercial enzyme-linked immunosorbent assay (ELISA) systems using recombinant forms of these proteins as target antigens (both from Immundiagnostik AG, Bensheim, Germany). Both assays were produced as described previously[8] and performed following the manufacturer's instructions. Each sample was tested in duplicate. Antibody concentrations were expressed in arbitrary units and the cut-off for both ELISAs was set at 18 U/mL.

Statistical analysis

As measured values did not follow normal distribution in any patient group, nonparametric methods were used for statistical analysis. The relationship between eTG and tTG ELISA values was assessed by the Spearman rank correlation. The Mann-Whitney U test for two independent samples was used to compare various patient groups. A test result was considered significant if the corresponding *P* value was less than .05.

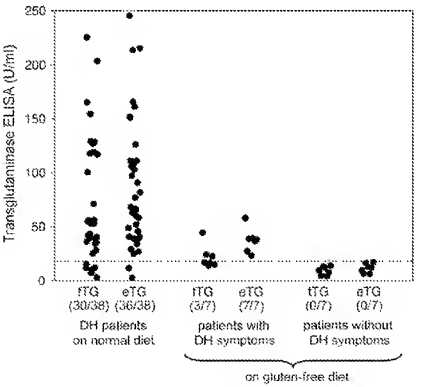

Fig 1. Scatter plot of autoimmune reactivity against epidermal transglutaminase (*eTG*) and tissue transglutaminase (*tTG*) in patients with dermatitis herpetiformis (*DH*) as determined by enzyme-linked immunosorbent assay (*ELISA*). *Interrupted line* corresponds to assay cut off (18 U/mL). *Numbers in brackets* are positive and total cases, respectively.

RESULTS

In all, 52 sera from patients with DH were studied for IgA reactivity against eTG and tTG by ELISA. The results of this analysis are summarized in Tables I and II.

In the group of 38 patients with DH on a normal diet, IgA antibodies to eTG were detected in 36 (95%) and those to tTG in 30 (79%) cases. Seven sera (18%) contained antibodies to eTG but not to tTG, whereas one serum showed exclusively tTG-specific reactivity. In one further patient, no reactivity against either TG was detected (Fig 1). IgA reactivities against eTG and tTG correlated with each other ($P = .017$). The presence of GSE was confirmed by intestinal biopsy

specimen in 18 of the 38 patients. Differences in eTG- and tTG-specific antibody levels between patients with DH, whose GSE was confirmed by a small intestinal biopsy specimen, and those for whom intestinal biopsy data were not available, were not significant.

None of 7 patients without DH lesions on a GFD was positive for reactivity against eTG or tTG. In the subgroup of 7 patients showing DH symptoms on a GFD, all had elevated antibodies to eTG, whereas antibodies to tTG were normal in 3 patients (Fig 1). One of these patients (patient 7) developed DH while on a GFD administered for GSE. He demonstrated autoantibodies to eTG but not to tTG.

DISCUSSION

The eTG has been demonstrated to be a preferential target of IgA antibodies in patients with DH that is recognized by both antibodies specific only for eTG and tTG-specific antibodies cross-reactive with eTG.[8] Previous studies on the sensitivity of anti-eTG antibodies for the primary diagnosis of DH yielded conflicting results. Although Sárdy et al[8] reported a sensitivity of 92%, Heil et al[12] and Hull et al[13] found anti-eTG antibodies in only 45% and 52% of patients with untreated DH, respectively. Interestingly, the sensitivity of the tTG ELISA was higher than that of the eTG ELISA in the series of Sárdy et al[8] and Heil et al,[12] whereas Hull et al[13] found the eTG ELISA to be a better tool for the diagnosis of DH. In the current study, we examined a large group of previously untreated patients with DH, consuming normal diet, and found eTG-specific antibodies in 95% of the sera. Importantly, the eTG ELISA identified 7 untreated patients with DH, who were negative for anti-tTG antibodies, whereas only one patient showed tTG- but not eTG-specific antibodies. The reasons for discrepant sensitivity values reported in the past are unclear, as all investigations used the same ELISA system as in the current study. Our results suggest that detection of IgA antibodies against eTG is a highly sensitive test for the primary diagnosis of DH and that it may be superior to the tTG assay in some cases. Although direct immunofluorescence microscopy of perilesional skin remains the gold standard for the definitive diagnosis of DH, eTG ELISA may be considered as a less invasive and less expensive screening test for ruling out DH.

Antibodies to tTG and eTG are known to decrease on a GFD and are, therefore, regarded as a useful indicator of patient adherence to diet.[3,5,8] We examined anti-eTG and anti-tTG antibody levels in 14 patients with DH taking a GFD. As expected, in 7 patients who had been able to discontinue suppressive dapsone therapy while on a GFD, no tTG- or eTG-specific antibodies were found. The remaining patients fell into two different groups. Three patients demonstrated both anti-tTG and anti-eTG antibodies, suggesting that they unintentionally continued consuming small amounts of gluten, whereas 4 further patients showed eTG-specific but not tTG-specific reactivity in their serum. One of these latter patients developed DH after being treated with a GFD for GSE during a period of 2 years. Interestingly, rare cases of DH, developing in patients with GSE despite a strict GFD, have been reported in the literature.[14-16] In our patient, at the time of GSE diagnosis, anti-tTG antibodies were found to be elevated and disappeared completely after 6 months of a GFD. When the patient was given a diagnosis of DH 18 months later, only anti-eTG but not anti-tTG antibodies were found. Thus, a subgroup of patients with GFD-resistant DH may demonstrate exclusive reactivity against eTG but not tTG. One may speculate that in these patients anti-tTG antibody production originally triggered by gluten is lost as a result of a GFD, whereas anti-eTG antibody production continues even in the absence of gluten exposure and after several years' results in DH manifestation. In addition, these observations further confirm that eTG is the principal autoantigen in DH.

In summary, we demonstrate that antibodies to eTG are the most sensitive serologic marker for the diagnosis of DH in patients on a normal diet or a GFD. Further efforts should focus on the development of a serologic test system for selective detection of eTG-specific antibodies without cross-reactivity to tTG. This antibody population seems to be particularly relevant for the pathogenesis of DH.

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